Original Research

Antioxidant Effects of Zinc Supplementation in Tunisians with Type 2 Diabetes Mellitus

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Objective: To determine the effects of zinc (Zn) supplementation on oxidative stress in persons with type 2 diabetes mellitus (type 2 DM).

Design: Tunisian adult subjects with HbA1c >7.5% were supplemented for six months with 30 mg/day of Zn as Zn gluconate or placebo. The effects of supplementation on plasma zinc (Zn), copper (Cu), urinary Zn, plasma thiobarbituric acid reactive substances (TBARS), Cu-Zn superoxide dismutase (SOD) and glutathione peroxidase activities (GPX) in red blood cells, blood lipids and lipoproteins, HbA1c and fasting glucose were measured at the beginning of the study and after three and six months.

Results: At the beginning of the study, more than 30% of the subjects exhibited plasma Zn values less than the normal minimum of 10.7 μmol/L, whereas levels of plasma Cu and antioxidant RBC Cu-Zn SOD and GPx enzyme activities were in the normal ranges. Oxidative stress, monitored by plasma TBARS, was increased in individuals with diabetes compared with healthy Tunisian subjects (3.32 $\pm$ 0.05 μmol/L *vs.* 2.08 $\pm$ 0.04 μmol/L) and an inverse correlation was found between Zn plasma levels and plasma TBARS. After three and six months of Zn supplementation, all of the subjects exhibited plasma Zn values greater than 10.7 μmol/L. There was a decrease of plasma TBARS in Zn supplemented group after six months (15%) with no significant changes in the placebo group. Supplementation did not alter significantly HbA1c nor glucose homeostasis. No adverse effects of Zn supplementation were observed on Cu status or HDL cholesterol.

Conclusions: These data suggest the potential beneficial antioxidant effects of Zn supplementation in persons with type 2 DM. These results are particularly important in light of the deleterious consequences of oxidative stress in persons with diabetes.

INTRODUCTION

Development of diabetic complications has been hypothesized to be accelerated by generation of free radicals in cells and tissues [1–2]. In diabetes, oxidative stress is due in part to an increased production of plasma free radical concentrations and a sharp reduction in antioxidant defenses [3]. Among the causes of enhanced free radical production, hyperglycemia [4], hyperinsulinemia and/or insulin resistance [5] play major roles, and it may be postulated that oxidative stress represents the common pathway through which hyperglycemia and insulin resistance induce depressed insulin action [6–7]. Oxidative stress in persons with diabetes is also related to decreased antioxidant defenses [8]. Several reports underlie the alterations of antioxidant micronutrient status in subjects with type 1 or 2 DM [9–12]. Antioxidant treatments, such as vitamin E

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[13–14], vitamin C [15] and lipoic acid [16], are able to improve insulin action. Among the trace elements, chromium has been shown to improve insulin function [17] and antioxidant status [18]. Zinc also is involved in diabetes, and metabolic disorders of diabetes are associated with a depleted zinc status [19–20]. Correction of Zn deficiency in subjects with type 1 DM leads to decreased lipid peroxidation [21] and improvements in glucose homeostasis [22]. Consequently, considering the possible modulating effects of zinc on insulin sensitivity and its antioxidant functions, we postulated that a restored Zn status in individuals with type 2 DM might counteract the deleterious effects of oxidative stress and help to prevent complications associated with diabetes.

The study was conducted under the auspices of the United Nations Educational Scientific and Cultural Organization, UNESCO, in Tunisia, where the incidence of type 2 DM is approximately 10%, with a high incidence of oxidative complications such as retinopathies, glomerulopathies and vascular complications. The aim of the study was to investigate the effects of Zn supplementation on oxidative stress in persons with type 2 DM.

MATERIALS AND METHODS

Subjects

Volunteers were adult males and females, 48 to 63 years of age, with diabetes for at least five years exhibiting fasting glucose >8 mmol/L and HbA1c $>7.5\%$. Key exclusion criteria included pregnant and lactating women, persons receiving trace element supplements in the previous three months, persons with gastric or diuretic treatment, patients with acute renal failure (creatinine >120 μmol/L) and patients with a recent surgery or acute infection. Subjects were enrolled from June to March from the Departments of Endocrinology of Sfax and Monastir Hospitals. The study received the agreement of the Tunisian Health Department and approval of the United States Department of Agriculture Research Service Human Studies Review Board. Subjects were informed of the purposes of the study, were free to ask questions throughout the study and signed an informed consent form witnessed by one of the investigators.

The study design was randomized, double blind and placebo controlled. Subjects, $n = 56$, were divided randomly into two groups and supplemented daily with either 30 mg of Zn as Zn gluconate or placebo. Zinc gluconate and placebo capsules (stearate of magnesium, 6 mg, silicon dioxide, 6 mg, cornstarch 28 mg and lactose, 200 mg) were provided by Labcatal Pharmaceutical (7 Rue Roger Salengro, 92541 Montrouge Cedex, France). Each month, the volunteers received the daily doses for one month and they were asked to return the non-used supply after one month to help measure their compliance. All the subjects reported in this paper completed the study. The compliance of the subjects participating in this study was 87%. Subjects were also asked questions regarding any possible side effects and degree of compliance. A group of 60 healthy Tunisian adults, age and gender matched, composed the reference group for plasma TBARS.

Analysis

Blood samples were drawn after an overnight fast at the beginning of the study and after three and six months of daily supplementation. Urine samples were collected each of the three times in four-liter brown plastic containers (Fisher Scientific, Pittsburgh, PA). Aliquot samples were dispensed into Falcon polypropylene tubes (Falcon, Oxnard, CA) and stored at -20°C. All the samples were run prior to the breaking of the code, which was not available to the investigators until completion of all the samples. Urinary Zn was determined by flame atomic absorption spectrometry using a Perkin-Elmer 5000 spectrometer on acidified urine samples. Plasma Zn and Cu were determined by flame atomic absorption spectrometry [23–24]. Red blood cell Cu-Zn SOD activity was evaluated by the method of Marklund and Marklund [25]. SeronormR Trace Element (Nycomed, Oslo, Norway) and an in-house pool of human erythrocytes and plasma were used as internal quality controls. Plasma TBARS were determined as described [26], using the fluorimetric determination of malondialdehyde (MDA)-TBA (thiobarbituric acid) complex after extraction with n-butanol (Sobioda MDA fluorimetry kit, Grenoble, France). Since the plasma MDA fluorimetric determination may also form a variety of chromogens other than the MDA-TBA adduct by reacting with substances such as amino acids or sugars, we verified every fifth sample at entry and every fourth sample at three and six months, using the TBA test followed by HPLC separation as described by Richard *et al.* [27]. The correlation coefficient between the two methods was 0.85 ($p < 0.001$). Samples verified by HPLC were representative of all the groups. Lipids, lipoproteins, HbA1c, fasting blood glucose and glutathione peroxidase were measured using routine laboratory methods.

Statistics

Statistical analyses of the data were performed by analysis of variance. Individual means comparisons were identified with Duncan's Multiple Range Test (SAS, SAS Institute, Cary, NC). Statistical significance was set at $p < 0.05$. Values are mean $\pm$ SEM.

RESULTS

As shown in Table 1, at the beginning of the study, the two groups were similar based upon age, duration of diabetes, weight, BMI, fasting glucose, HbA1c, insulin, cortisol, total cholesterol and HDL cholesterol. Following supplementation,

Table 1. Baseline Characteristics of Subjects

	Zinc (27)	Placebo (29)
Age	51.5 ± 1.62	55.5 ± 1.43
Duration of diabetes (months)	69.5 ± 6.11	72.5 ± 6.32
Weight (kg)	74.5 ± 0.33	77.3 ± 0.35
BMI (kg/m^2)	28.9 ± 0.15	29.6 ± 0.15
Fasting Glucose (mmol/L)	11.1 ± 0.07	11.4 ± 0.08
HbA1c (%)	8.87 ± 0.37	8.96 ± 0.24
Insulin (pmol/L)	102 ± 9	108 ± 8
Cortisol (nmol/L)	411 ± 22	436 ± 28
Total Cholesterol (mmol/L)	5.61 ± 0.18	4.97 ± 0.20
HDL Cholesterol (mmol/L)	1.19 ± 0.05	1.11 ± 0.05

Number in parenthesis denotes number of subjects per group.

there were no statistical changes in any of these variables. HbA1c decreased from 8.9 ± 0.4 to 7.7 ± 0.3% following six months of Zn supplementation, but decreases were not significant at $p < 0.05$.

At the beginning of the study, plasma Zn and Cu were similar in the two groups (Table 2). More than 30% of the subjects exhibited plasma Zn levels below the cut off of 10.7 μmol/L, which is considered to be at risk of Zn deficiency. After three months of daily Zn supplementation, none of the subjects displayed plasma Zn values below 10.7 μmol/L. Plasma Zn increased from 11.3 ± 0.18 to 12.5 ± 0.19 then to 13.7 ± 0.20 μmol/L in the group receiving supplemental Zn for three and six months, respectively (Table 2). There were no significant differences in the placebo group, and the incidence of Zn deficiency was similar at the beginning and end of the study. Plasma Cu levels of the subjects with diabetes were similar to those of the controls and were not altered by Zn supplementation (Table 2). Urinary Zn losses were also similar at the onset of the study and were not altered by supplementation (Table 2).

Plasma TBARS in subjects with type 2 DM (Table 3) were significantly higher than those measured in control adult Tunisians (2.08 ± 0.04 μmol/L). An inverse correlation was found between plasma Zn and the levels of TBARS (Fig. 1) with a negative correlation coefficient of 0.48. This decreased to −0.23 after six months of zinc supplementation (Fig. 2). After three months of supplementation, a nonsignificant decrease in plasma lipid peroxidation was observed, and this decrease became significant after six months ($p < 0.001$) (Table 3). Plasma TBARS decreased 13.6% in the group receiving Zn compared with the placebo group, and there were no

Table 2. Zn Supplementation Effects on Plasma Zn, Cu and Urinary Zn

	Zinc	Placebo
Plasma Zn (μmol/L)		
Initial	11.3 ± 0.18	11.4 ± 0.19
3 months	12.5 ± 0.19	11.7 ± 0.19
6 months	13.7 ± 0.20**	11.8 ± 0.19
Plasma Cu (μmol/L)		
Initial	16.30 ± 0.05	16.8 ± 0.07
3 months	16.9 ± 0.05	17.2 ± 0.07
6 months	17.4 ± 0.07	18.0 ± 0.06
Urine Zn (μg/mg Ct)		
Initial	0.76 ± 0.09	0.93 ± 0.14
6 months	0.88 ± 0.09	0.88 ± 0.07

** Significant effect.

Table 3. Plasma TBARS and Antioxidant RBC Cu-Zn SOD and Se GPx Activity after Three and Six Months of Supplementation

Group	Zinc	Placebo
TBARS (μmol/L)		
Initial	3.32 ± 0.06	3.18 ± 0.05
3 months	3.03 ± 0.06	3.10 ± 0.05
6 months	2.87 ± 0.08***	3.04 ± 0.07
Cu-Zn SOD RBC (U/mg Hb)		
Initial	1.20 ± 0.02	1.20 ± 0.02
3 months	1.28 ± 0.05	1.31 ± 0.05
6 months	1.20 ± 0.02	1.25 ± 0.02
RBC Glutathione Peroxidase (U/gHb)		
Initial	39.8 ± 1.5	41.1 ± 2.6
3 months	44.7 ± 1.5	45.0 ± 2.7
6 months	44.7 ± 1.6	46.2 ± 2.6

*** Significant effect of supplementation, $p < 0.001$.

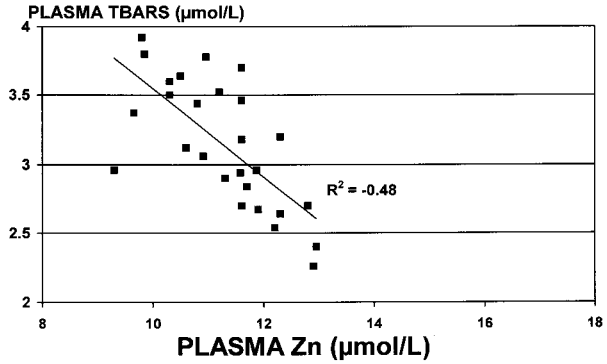

Fig. 1. Correlation of plasma TBARS with plasma zinc after the placebo period.

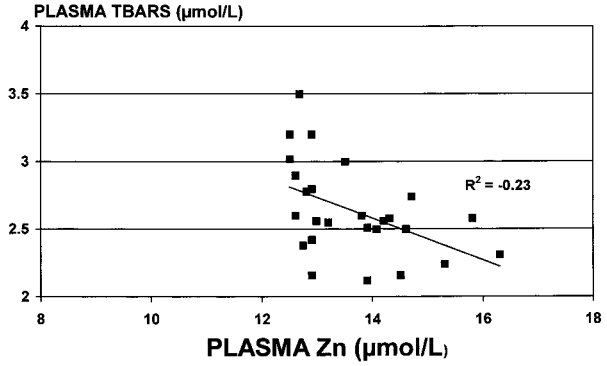

Fig. 2. Correlation of plasma TBARS with plasma zinc after six months of supplemental zinc.

detectable changes in the placebo group. The antioxidant activity of Cu-Zn SOD was not altered (Table 3) and was comparable to those previously described in European adults [28] or Algerian adults [29] using the same method of determination. RBC glutathione peroxidase was also unchanged (Table 3), and there were no decreases in plasma Cu and HDL cholesterol values due to zinc supplementation.

DISCUSSION

In this work, zinc supplementation caused a reduction in the lipid peroxidation monitored by plasma TBARS in persons with type 2 DM. The results of this study corroborate our previous data demonstrating that, in individuals with type 1 DM receiving similarly 30 mg of Zn as Zn gluconate for three months, there were also decreased lipid peroxidation and improvements in antioxidant status [21–22]. In persons with diabetes, the vulnerability to oxidative damage may be partly attributed to lower antioxidant micronutrients. Impairments of Zn status have been reported as risk factors in the progression of diabetes [30]. The potential antioxidant effects of Zn in diabetes could be related to several mechanisms [31]. Zn plays a structural role in the maintenance of Cu-Zn SOD structural integrity [32]. Zn metallothionein complexes in the islet cells provide protection against immune-mediated free-radical attack [33], and Zn could act also in protecting sulfhydryl groups against oxidation and participate in the inhibition of the free radical production in the Haber Weiss cycle by competing with transition metals [34]. By preventing proteins from oxidation, Zn could contribute to SH group stabilization and thus might influence transcription factors such as p53 [35]. Zinc also functions as a cofactor for caspases [36], enzymes of nucleic acid catabolism, and Zn depletion results in cellular death by apoptosis [37].

Some investigators have also speculated that zinc supplementation could improve insulin sensitivity in type 2 DM [38]. As suggested by Preuss [39], there may be improvements in insulin sensitivity that are associated with improved antioxidant status. Insulin resistance is associated with increased lipid peroxidation and free radical formation [40]. Improvements in antioxidant variables may be more sensitive than the changes in circulating insulin and glucose concentrations. At the onset of our study, plasma TBARS were significantly elevated in individuals with type 2 DM compared with apparently healthy control subjects (3.32 ± 0.05 *vs.* 2.08 ± 0.04 μmol/L), thus confirming that lipid peroxidation increases in persons with diabetes [41–43]. These elevated levels of TBARS could result from the hyperglycemic state in relation with autooxidation of plasma glucose and other small autooxidizable molecules [6–7] and are associated with poor metabolic control of plasma glucose [44–45].

Zinc has numerous potential targets to modulate insulin activity. In rats, zinc deprivation led to the demonstration that zinc plays a role in insulin synthesis and activity [46]. We demonstrated previously that zinc depletion from insulin decreases its activity in rats [47]. Nutritional zinc supplementation improves fasting insulinemia and glycemia in genetically obese ob/ob mice [48]. In humans, glucose tolerance was reported to be improved in a patient with pancreatic diabetes and clinical features associated with zinc deficiency when given oral zinc sulfate [49]. We also observed previously the improvements of zinc status in ketotic insulin-dependent diabetic subjects under insulin therapy [22].

In this study, subjects who received 30 mg/day of zinc as gluconate displayed reduced lipoperoxidation. Since high serum zinc concentrations resulting from high zinc supplementation (220 mg/day) in individuals with diabetes might block peripheral insulin receptors [50] and lead to decreased glucose tolerance, it is important to stress that a moderate zinc supplementation as we used in this study leads to no detectable adverse effects.

CONCLUSIONS

These data demonstrate potential beneficial antioxidant effects of Zn supplementation in persons with type 2 DM. The mechanism of this action could be due to the antioxidant effects of zinc, especially in protecting SH groups, but also the modulating effect of zinc on insulin sensitivity cannot be ruled out. These results are particularly important in light of the deleterious consequences of oxidative stress in persons with diabetes. Increased intake of zinc, in addition to other nutritional and pharmacological treatments, may be important in the delay and/or prevention of the complications of diabetes.

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